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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 12 March 1999 with an application for Letters Patent number 334627 made by CHRISTIAN JOHN COOK.

I further certify that pursuant to a claim filed on 26 January 2000 under Section 24(1) of the Patents Act 1953, a direction that the application proceed in the name of THE HORTICULTURE AND FOOD RESEARCH INSTITUTE OF NEW ZEALAND LIMITED by virtue of a deed dated 13 January 2000.

Dated 16 March 2000.

Nevilie Harris
Commissioner of Patents

334627

NEW ZEALAND

PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

INCREASING EFFICACY OF THERAPEUTIC AGENTS AND ANIMAL GROWTH

I, CHRISTIAN JOHN COOK, a New Zealand citizen of 70 Nevada Road, Hamilton, New Zealand do hereby declare this invention to be described in the following statement:

INCREASING EFFICACY OF THERAPEUTIC AGENTS AND ANIMAL GROWTH

FIELD OF THE INVENTION

This invention relates to methods and compositions for enhancing efficacy of therapeutic agents and animal growth.

BACKGROUND TO THE INVENTION

Animals are susceptible to both external and internal parasitic infection and disease. This is especially true in an agricultural environment where a high concentration of animals means that infection and reinfection can easily occur. Parasitic and disease loads on livestock are known to be responsible for a number of conditions such as poor growth, anaemia, scouring, decreased milk production, indigestion, poor feed conversion, depression and premature death. These conditions hamper meat production and quality and have a detrimental economic impact on both the farmers and the industry in general.

In order to address this problem, therapeutic agents including vaccines, antibiotics, anthelmintics (also known as anthelminthics) and other anti-pathogenic agents have been used to control disease and the numbers of parasites in and on livestock. Therapeutic agents come in a number of forms, including drenches, pour-ons, wipe-ons, injectables, oral dosages or slow release compositions and are used to prevent, control or eliminate internal and external parasites and disease. Therapeutic agents and especially vaccines, antibiotics and anthelmintics are now well recognised as essential to healthy livestock growth.

However, therapeutic agents have disadvantages in that targeted organisms have been found to be developing resistance. One method used to tackle the increase in resistance has been to increase the number of doses and amounts of the agents administered to livestock.

It has also been shown that increasing agent use can, in and of itself, cause further resistance to the agents to develop.

As a result of increased agent usage, the costs of achieving the same disease or parasitic control per head of livestock escalate because of both an increase in labour and an increase in the amount of agent needed. A further problem encountered with more

frequent use of some therapeutic agents is the build up of chemical residue within livestock, making the meat worth less and, in some cases, not fit for human consumption. Animals also suffer an increase in handling stress due to the need for increased handling to administer the agents more frequently.

It is also known in the art that handling stress is a contributory factor in livestock weight loss. This, in turn means that livestock use more pasture for less of an economic return. This problem has been found to be particularly acute in animals which have a propensity to be easily stressed.

Clearly, many of these disadvantages could be addressed if animal stress levels could be reduced and the efficacy of the agents administered could be increased.

The applicants have now surprisingly found that selected antistress agents when administered to an animal can promote growth of that animal beyond what might be anticipated from reduction in stress alone.

Moreover, the applicants have also unexpectedly found that the selected antistress agents when combined with therapeutic agents increase the efficacy of the therapeutic agent in a synergistic manner.

An object of the present invention is to provide methods and compositions for enhancing efficacy of therapeutic agents and animal growth which go some way to overcoming the above drawbacks or at least provide the public with a useful choice.

SUMMARY OF THE INVENTION

A first aspect of the present invention provides a method for promoting animal growth, the method comprising administering at least one therapeutic agent to an animal and reducing the stress experienced by the animal.

In one embodiment, reduction in stress experienced by the animal is achieved by reducing physical causes of stress.

In an alternative embodiment, reduction in stress is achieved by administering an antistress agent to the animal.

Animal growth promotion is preferably by weight gain.

In accordance with a further aspect, the present invention provides a method for enhancing the efficacy of a therapeutic agent, the method comprising the co-administration of at least one therapeutic agent and at least one antistress agent to an animal.

Co-administration encompasses both concurrent and sequential administration. Conveniently, the antistress agent and therapeutic agent are administered in the form of a combined composition.

Accordingly, in a further aspect, the present invention provides a therapeutic composition comprising at least one therapeutic agent and at least one antistress agent.

In one embodiment, the therapeutic composition is formulated as a slow-release composition.

Preferably, the therapeutic composition is an anthelmintic composition.

The invention extends to the use of antistress agents as adjuvants for therapeutic agents and compositions.

The invention also extends to the use of antistress agents as protective or recovery aiding agents, and methods of treatment using same.

A further aspect of the present invention contemplated is the use of antistress agents as animal growth promoters. Methods of promoting animal growth by administering the antistress agents also form part of the invention.

In a still further aspect, the invention provides a method of treating an animal, the method comprising administering a therapeutic composition of the invention to said animal.

In a preferred method of treatment, the animal is an animal infected with helminths and the therapeutic composition is an anthelmintic composition.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a bar graph with a superimposed line graph depicting the level of faecal egg estimates on the bar graph and animal growth on the line graph for chronically stressed animals and a control group.

Figure 2 is a line graph depicting the level of faecal egg estimates on the first Y axis and animal growth on the second Y axis line graph for acutely stressed animals and a control group.

Figure 3 is a bar graph with a superimposed line graph depicting the level of faecal egg estimates on the bar graph and animal growth on the line graph for chronically stressed animals treated with metyrapone.

Figure 4 is a line graph showing the effects of administration of metyrapone on acutely and chronically stressed animals.

Figure 5 is a bar graph showing the effect on animal growth of metyrapone.

DETAILED DESCRIPTION OF THE INVENTION

As discussed above, the applicants have surprisingly found that selected antistress agents, when administered to animals have unexpected effects in promoting animal growth and increasing the efficacy of therapeutic agents. In an extension of this finding, the applicants have found that animal growth may be surprisingly promoted by administering one or more therapeutic agents to an animal and reducing the stress experienced by same.

Accordingly, in a first aspect, the present invention provides a method for promoting animal growth, the method comprising administering at least one therapeutic agent to an animal and reducing the stress experienced by the animal.

Animals susceptible to treatment according to the invention include humans and other animals. Other animals may encompass pets and livestock including cats, dogs, birds, pigs, sheep, fish mink, deer, goats, cattle, horses, chickens and turkeys, but are not limited thereto. Best results are likely to be achieved with animals which are prone to high levels of stress.

Generally, the animals to be treated are sheep, deer, goats, cattle or pigs.

The term "therapeutic agent" as used herein refers broadly to agents useful in the treatment or prevention of disease or infestation in an animal otherwise useful in promoting animal growth and well being. Included in the term are vaccines, antibiotics,

anthelmintics, other anti-pathogenic agents, growth promoters, vitamin and mineral supplements but are not limited thereto. The term "therapeutic composition" is to similarly understood as broadly defined.

A very broad range of therapeutic agents are known in the art. Vaccines and antibiotics are described for example in *The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal and Antiviral Drugs*, A. Kucers, S.M. Crowe, M.L. Grayson, J.F. Hoy: 5th Edition Butterworth Heinemann 1997; and *Equine Drugs and Vaccines*, E. Kelton and T. Tobin, Breakthrough Pub. 1995; and *Vaccines for Veterinary Applications*, A.R. Peters (Ed.) 1993 all incorporated herein by reference.

Anthelmintics are preferred therapeutic agents.

A broad range of anthelmintics suitable for use in the methods are known in the art. A general reference text is Chemotherapy of Parasitic Disease; William Campbell, Plenum Publishing 1986 (incorporated herein by reference).

Suitable classes of anthelmintics which can be used include those active against cestodes, trematodes, nematodes and acanthocephala. The compounds may be selected from the group comprising simple heterocyclic compounds, benzimidazoles, imidazothiazoles, tetrahydropyrimidines, organophosphates, macrocyclic lactones, arsenicals and anticestodal drugs.

More preferably, suitable anthelmintic compounds are selected from the group comprising piperazine, diethylcarbamazine citrate, thiabendazole, fenbendazole, albendazole, oxfendazole, oxibendazole, febantel, tetramisole (levamisole, levamisole hydrochloride), pyrantel tartrate, pyrantel pamoate, morantel tartrate, dichlorvos, milbemycin oxime, eprinomectin, moxidectin, N-butyl chloride, toluene, hygromycin B, sodium arsenamide sodium, melarsomine, praziquantel, epsiprantel, clorsulon, triclabendazole, diazinon, benzimidazole, salicylamide, isoquinoline and cyromazine amongst others.

Preferred commercially available anthelmintics for use in the invention include Fasinex®, Soforen®, Endex®, Combinex®, Parifal®, Neocidol®, Acutak®, Dimpygal®, Nucidol®, Sarnicida®, Topclip®, Sentinel®, Vetrazin®, Avermectin®, Ivermectin® and Doramectin® but are not limited thereto. Combinations of two, three or more anthelmintics with the same or different anti-pest activity are also contemplated.

The therapeutic agents referenced above include drenches, pour-on formulations,

injectables, oral dosage forms and slow release formulations, amongst others. It will therefore be appreciated that administration of the agent orally, parenterally, topically and by injection is contemplated. Single and multiple dosing regimes are contemplated. Multiple dosing regimes may comprise administration of two or more agent doses to different sites on, or by different routes of administration to, an animal at the same time.

In one embodiment, multiple dosing regimes may comprise administration of two or more doses of agents to different sites on an animal over a period of time covering days, weeks or months.

In a preferred anthelmintic treatment regime, animals are dosed every three to four months by a combination of pour-on, injection and oral treatments.

Dosing, and dosages will be according to manufacturers instructions or as otherwise known in the art. For example, for anthelmintics where nematode counts exceed 600 dosing is generally recommended.

The present applicant has also found that the animal's state of stress, both acute and chronic, can contribute to the lasting efficacy of either a pour-on, oral or an injectable agent. Animals that have a high acute level of stress, at the time of application, or alternatively a low to high chronic stress load for some time prior to, or after, application show a lowered efficiency from the dosage and show a quicker re-infestation.

The stress undergone by the animal may be psychological stress or physical stress. Psychological stresses include restraint, handling and novelty stress. Physical stresses include hunger, thirst, fatigue, injury, trauma, surgery or thermal extreme stress. The stress may be also be chronic or acute.

The stress experienced by the animal may also be characterised as being of short duration or alternatively of long duration. In the case of short duration stress reduction, the stress reduction preferably takes place before the administration of the therapeutic agent, but can be after the administration of the agent. In the case of long duration stress reduction, the stress reduction is preferably of an order of at least the time between any agent administrations to the animal.

In one embodiment, stress reduction can be achieved by reducing physical causes of stress, preferably by way of reduced handling of the animals. This can be achieved by reducing intervention with the normal living patterns of the animal. It may include reducing animal

(for example dog) and human interaction with the animals, limiting movements, shortening transport procedures and the like. However, physical stress reduction is not always practical.

Accordingly, in an alternative embodiment, stress reduction is achieved by administering at least one antistress agent to the animal.

The term "antistress agent" as used herein refers to compounds or compositions effective in reducing stress. This may be physiological or psychological stress or a combination thereof. Not included are agents which simply act as nutritional modifiers such as foodstuffs (for example, molasses and propylene glycol), or electrolytes. Accordingly, the antistress agents used herein are not simply nutritional modifiers but also physiological and/or psychological stress reducers. Any appropriate antistress compounds or compositions known in the art may be employed. The antistress agent is preferably long-acting, although short acting antistress agents are not excluded. In one embodiment, the antistress agent is formulated as a slow-release composition.

Suitable classes of antistress agents, including glucocorticoid inhibitors, corticotrophin releasing hormone inhibitors, ACTH inhibitors, cholecystokinin inhibitors, benzodiazepines, gamma amino butyric acid potentiators, anti-glutaminergics and serotonergics amongst others.

Preferred antistress agents include metyrapone, mifipristone, αCRH 9-41, proglumide, diazepam, allopregnanolone, dextromethorphan, zimelidine and paroxetine but are not limited thereto. Combinations of two, three or more antistress agents with the same or different activity are also contemplated for use herein. A particularly preferred antistress agent for use in the present invention is metyrapone. This compound acts to suppress some of the physiological and psychological stress responsiveness in an animal, including elevation of levels of glycocorticoid hormone cortisol .

Antistress agents may again be administered in a broad effective range. Appropriate dosage rates can be selected by the skilled reader according to known protocols for treating a variety of animals. Variation will occur based on the animal to be dosed, age, body weight and the like. Dosages within the range of 0.001 to 500 mg/kg liveweight are feasible. For metyrapone a preferred dosage range is 0.01 to 20 mg/kg liveweight.

Stressed animals that received this treatment showed approximately the same efficacy of anthelmintics as non-stressed animals and similar growth rates. This point is illustrated

in accompanying Figures 2 to 4.

In a second aspect, the present invention provides a method for enhancing the efficacy of a therapeutic agent, the method comprising the co-administration of at least one therapeutic agent and at least one antistress agent to an animal.

Co-administration encompasses both concurrent and sequential administration. For sequential administration, it is not critical whether the therapeutic agent or antistress agent is administered first. Sequential administration may occur over a period of minutes, hours, or days. However, concurrent administration is preferred.

For concurrent administration, the therapeutic agent and antistress agent are preferably formulated in the same composition as discussed below.

A further aspect of the present invention relates to a therapeutic composition comprising at least one therapeutic agent and at least one antistress agent. The therapeutic and antistress agent may be selected from any of the therapeutics and antistress agents or combinations thereof referenced above or otherwise known in the art. In a preferred composition, the therapeutic agent is an anthelmintic. In a particularly preferred composition, the anthelmintic is selected from Ivomec, Endex and levamisole with metyrapone.

The amounts of therapeutic agent(s) and antistress agent(s) in the composition may vary within a broad range, so long as effectiveness is maintained. Antistress agent(s) will generally be present in individual or combined form between 0.0001 to 99.999% of the composition.

As discussed above, the compositions of the invention can be formulated for oral, parenteral and topical administration or for injection.

To produce such formulations, the therapeutic compositions of the invention may further contain pharmaceutically or agriculturally acceptable carriers, diluents, excipients, disintegrators and binding agents and such other materials as are known in the art and customarily employed in such formulations. The compositions may further comprise preservatives, antioxidants, colourants, feedstuffs, nutrients, vitamins, lubricants, salts and other therapeutic agents. This list is illustrative rather than exhaustive of the components of the composition.

In one preferred embodiment, the composition is formulated as a slow-release composition, such as are known in the art. Slow release of the composition may conveniently be achieved through the use of boluses or time release capsules.

Examples of boluses contemplated by the invention are those as set out, for example in GB 2, 122,086, US 3,535,419 and US 5,720,972, which are incorporated herein by reference.

Using the methods and/or compositions of the invention, the applicants have found that selected antistress agents when combined with therapeutic agents or compositions increase the efficacy of the therapeutic agent beyond what would be expected for the agents acting alone. Efficacy is usefully measured either in terms of increased effectiveness or duration of effectiveness of therapeutic agent. The route of action may vary. For example, in the case of vaccines, the antistress agent may act to increase the antibody titre and hence effectiveness.

The use of these treatments or compositions can therefore reduce the total number of therapeutic treatments needed in a year for effective results. A particular advantage here is that more time may be provided for residue clearance. Alternatively, if the number of treatments remains the same then a corresponding increase in effectiveness of the therapeutic agents should be seen. This means that the effective amount of the therapeutic agent required is reduced.

A still further benefit is reduction in stress at any stage in an animal's life, including in a pen prior to slaughter, where stress can reduce overall meat yield and quality.

A further benefit is the promotion of animal growth demonstrated using the compositions and methods of the invention. This may comprise growth overall during the course of the animal's life or in a pen prior to slaughter. Animal growth in the context of the present invention is primarily measured in terms of weight gain, although other measures are not excluded.

In the experiments carried out to date, and detailed below, the applicants have also surprisingly found that the antistress agents when administered to an animal can promote growth of that animal beyond what might be anticipated from reduction in stress alone.

Accordingly, in a further aspect, the present invention provides the use of antistress agents as discussed above as animal growth promoters. Methods of promoting animal growth

by administering the antistress agents are similarly contemplated. The antistress agents to be administered comprise any of those agents referenced above. Administration protocols are similarly discussed above.

Conveniently, the antistress agents may be used in animal feedstuffs to achieve the growth promotion benefits. Examples of suitable feedstuffs include hay, silage, haylage, grain, cereals and chicory or any other animal feedstuff produced naturally or artificially manufactured.

The use of antistress agents as protective or recovery aiding agents before, during or after periods of physiological or physical stress as discussed above is also contemplated. The use of antistress agents in conjunction with surgery or trauma may be desirable. Examples of trauma include myocardial infarction and cerebrovascular accidents (strokes and brain haemorrhage) but are not limited thereto. That is, the antistress agent may have protective function, especially an organ protective function in the case of physical or psychological stress.

Methods of treating an animal to prevent or aid recovery from stress, particularly surgery or trauma, comprising administering one or more antistress agents alone or with one or more other therapeutic agents or compositions is therefore contemplated.

The present invention also extends to the use of antistress agents as adjuvants for therapeutic agents. The use may comprise the concurrent or sequential administration of one or more antistress agents with the therapeutic agent(s). Suitable agents and administration protocols are as discussed above.

In a still further aspect, the invention provides a method of treating an animal, the method comprising administering a therapeutic composition of the invention to said animal. In a preferred method of treatment, the animal is an animal infected with helminths and the therapeutic composition is an anthelmintic composition. Again, any of the compositions and administration regimes referenced above may be employed in this method of the invention.

The invention will now be described with reference to the following non-limiting examples.

In a series of experiments, sheep were divided into pairs from initial twins. All the animals were grazed together, on the same paddocks, from pairing through to the end of

the trial. They were thus exposed to the same potential parasite load and the same access to feed and water. The animals were also genetically similar (i.e at least one shared parent). Experiments were begun when the animal was 3 months of age.

Animals had ad libitum access to grazing, water and supplementary feed such that intake was never limited.

EXPERIMENT 1

One half of the pairings were rounded up once a week and run through a race (control group). Any normal farm maintenance that was needed was done at this time, so that the time that animals may have been held was variable from week to week but consistent across the entire group and with the experimental group (see below). Faecal samples were also collected and both total egg and nematode egg counts calculated, and visual inspection for ectoparasites made.

The other half of the pairings (chronic stress group) were exposed to the same handling three times a week (consistent with the single weekly procedure above) and in addition to this were run around a paddock by either a human or dog (alternating) for 10 minutes. In this manner a mild, chronic, handling stress was imposed on these animals. Races and handling facilities were cleaned between animal movements to avoid any risk of parasitic contamination.

Animals were subject to the same anthelmintic treatments consisting of three pour-on (Ivomec pour on, MSD 1 ml per 50kg), three oral (Endex, Novartis - 1ml per 5kg) and three injectable treatments (levamisole. 7.5 mg/kg) per year (every four months).
As can be seen from figure 1 parasitic load during the course of the year was far greater in the stressed group, and growth rate was less than the control group, and the duration of knockdown of parasitic numbers for the set anthelmintic dosage less.

EXPERIMENT 2

In a similar parallel experiment the control group was identical and the paired group to this control (acute stress) received identical treatment to the control group and in addition an acute stress one day prior to anthelmintic treatment. This consisted of chasing the group three times during the day with a dog for 15 min duration each time. The faecal egg count and ectoparasite assessment was the same in both groups at time of the first experimental anthelmintic treatment. Anthelmintic treatment was administered at the

same dosage as one of the four monthly treatments above and then faecal eggs counts followed for up to 6 months, with no further treatment. At average nematode counts of 600-700 treatment is recommended. It can be seen from figure 2 that the knock down time (time following the treatment in which egg counts were maintained below this acceptable number) was less in the acute stressed group. Again growth rate was less in the stressed group.

For both experiment 1 and 2 each group of animals were then crossed-over in experimental treatments (i.e. each group received the treatment of its counterpart). A similar treatment dependent effect on anthelmintic efficacy emerged.

EXPERIMENT 3

In a similar set of experiments to experiment 1, the animals in the chronic stress group also all received a chemical substance metyrapone (in an oral form at 5mg/kg live weight) at the time of anthelmintic treatment. This substance is known to suppresses some of the physiological stress responsiveness, including elevation of levels of the glucocorticoid hormone cortisol.

Animals that received this treatment showed the same efficacy of anthelmintics as the non-stressed groups and similar growth rates. Figure 3 illustrates this data.

EXPERIMENT 4

The acute versus control groups of experiment 2 were also repeated with the acute group receiving metyrapone as above. Again no differences were seen between the acute and control groups when metyrapone was administered.

EXPERIMENT 5

This was again a repeat of experiment 2 but also included the chronic stress group. One treatment was given including metyrapone to stressed (acute and chronic) animals and the time until nematode counts exceeded recommended number for dosing followed. With metyrapone treatments both chronic and acute stressed animals showed no differences to the control group (Figure 4).

EXPERIMENT 6

In this experiment two groups were set up as per the original control groups in experiment 1 (i.e no additional stresses) and were treated as per anthelmintics in this experiment (i.e 4 treatments per year). In addition one of these control groups received metyrapone (5mg/kg) at the time of anthelmintic treatment.

Growth rate over the year was compared. The control group that received no metyrapone showed a net gain of mean 12 kg and standard deviation 3 kg. The metyrapone treated group showed 18 kg mean and standard deviation of 2 kg. The results are shown in Figure 5.

It will be appreciated by those persons skilled in the art that the foregoing description is provided by way of example only and that the scope of the invention is not limited thereto.

CHRISTIAN JOHN COOK

By the authorised agents
A. J Park & Son
Per

Figure 1

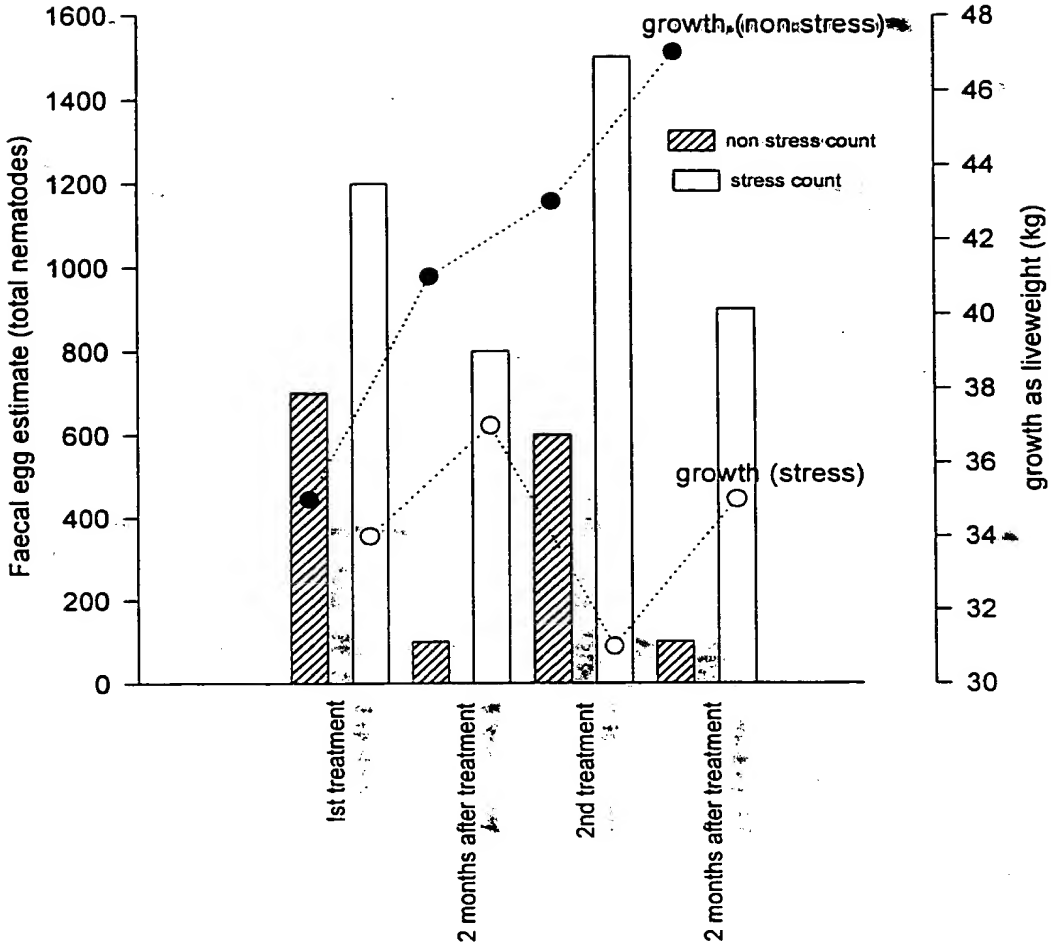

Faecal egg estimate (total nematodes)
1600
1400
1200
1000
800
600
400
200
0
growth (non-stress)
non stress count
stress count
growth (stress)
48
46
44
42
40
38
36
34
32
30
growth as liveweight (kg)
1st treatment
2 months after treatment
2nd treatment
2 months after treatment

Figure 2

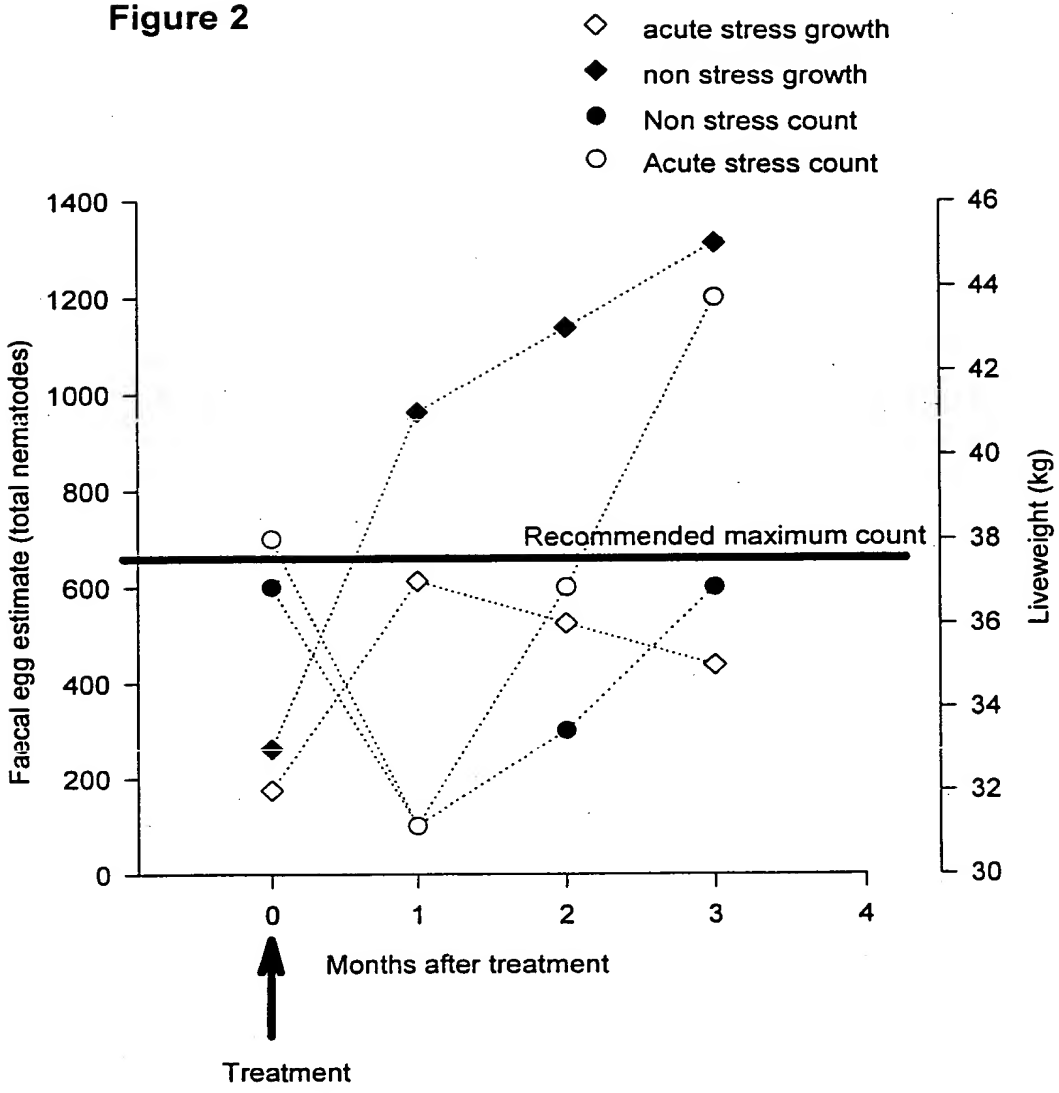
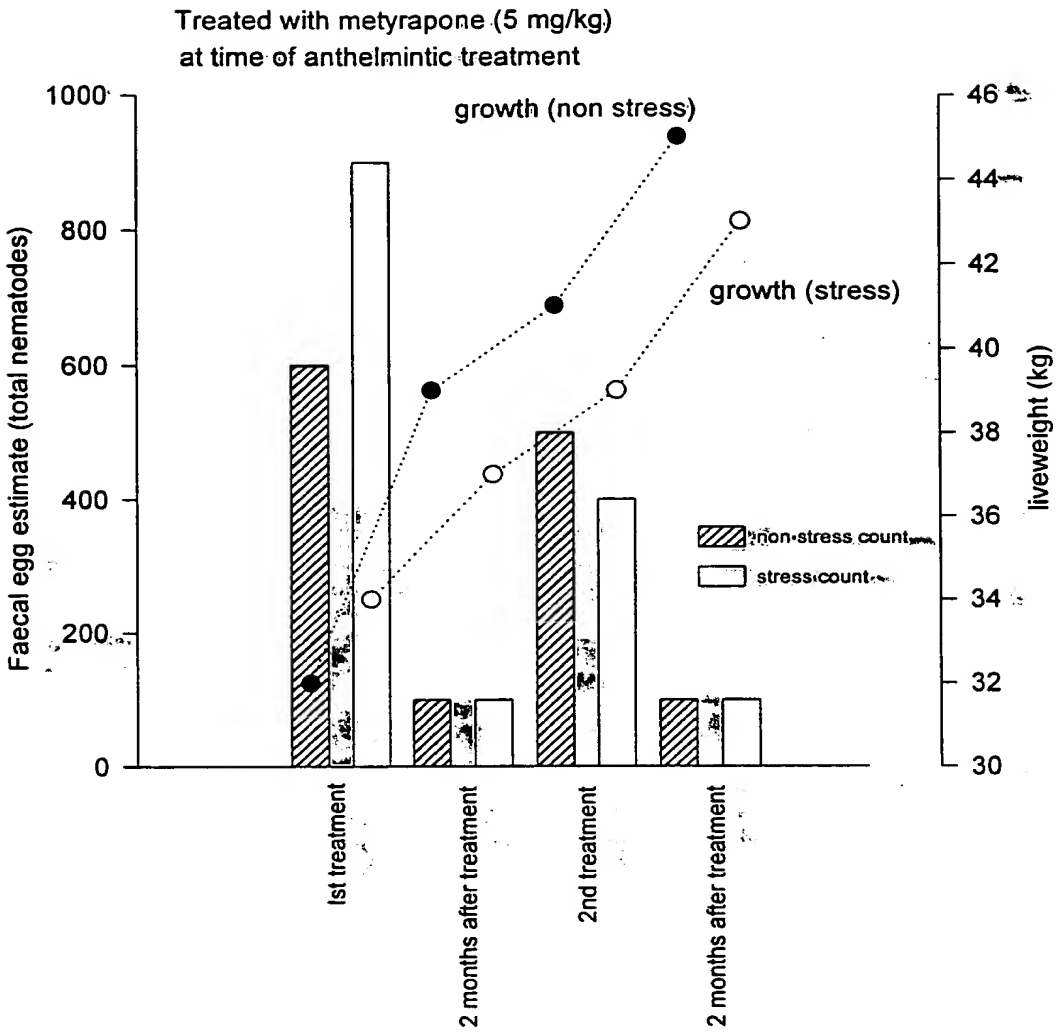

acute stress growth
non stress growth
Non stress count
Acute stress count
Faecal egg estimate (total nematodes)
Liveweight (kg)
Recommended maximum count
Months after treatment
Treatment
0
200
400
600
800
1000
1200
1400
30
32
34
36
38
40
42
44
46
0
1
2
3
4

Figure 3


Treated with metyrapone (5 mg/kg)
at time of anthelmintic treatment
growth (non stress)
growth (stress)
non-stress count
stress count
Faecal egg estimate (total nematodes)
liveweight (kg)
1000
800
600
400
200
0
46
44
42
40
38
36
34
32
30
1st treatment
2 months after treatment
2nd treatment
2 months after treatment

Figur 4

Metyrapone treatment with anthelmintic treatment

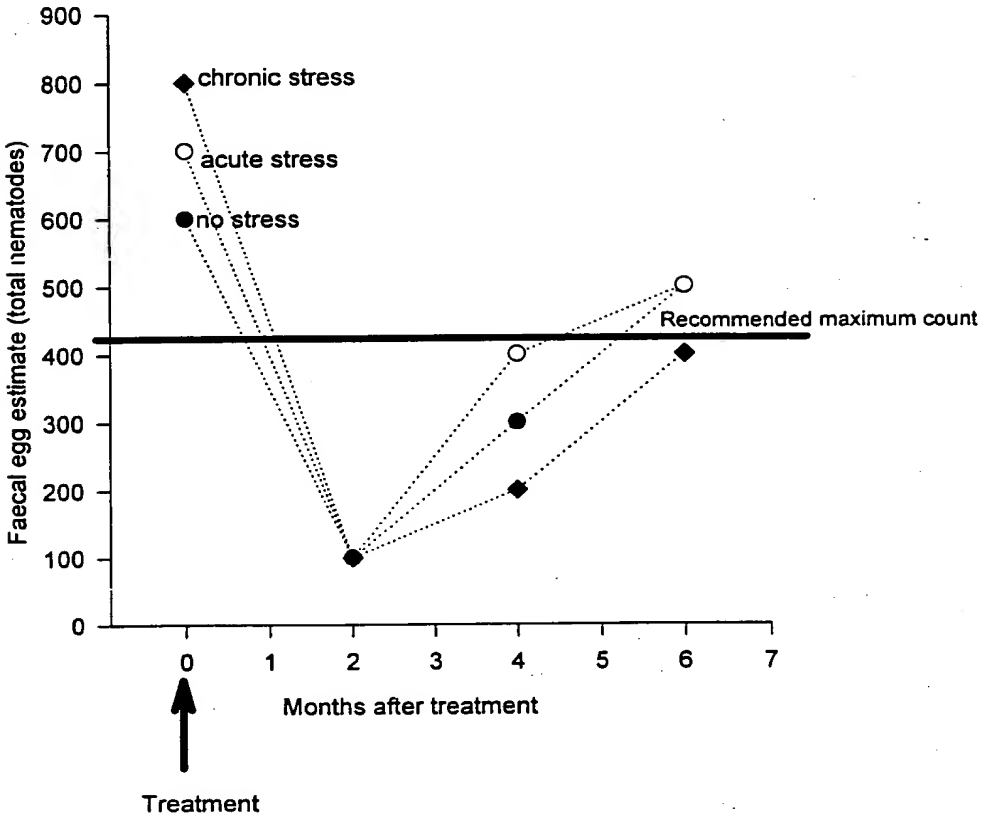
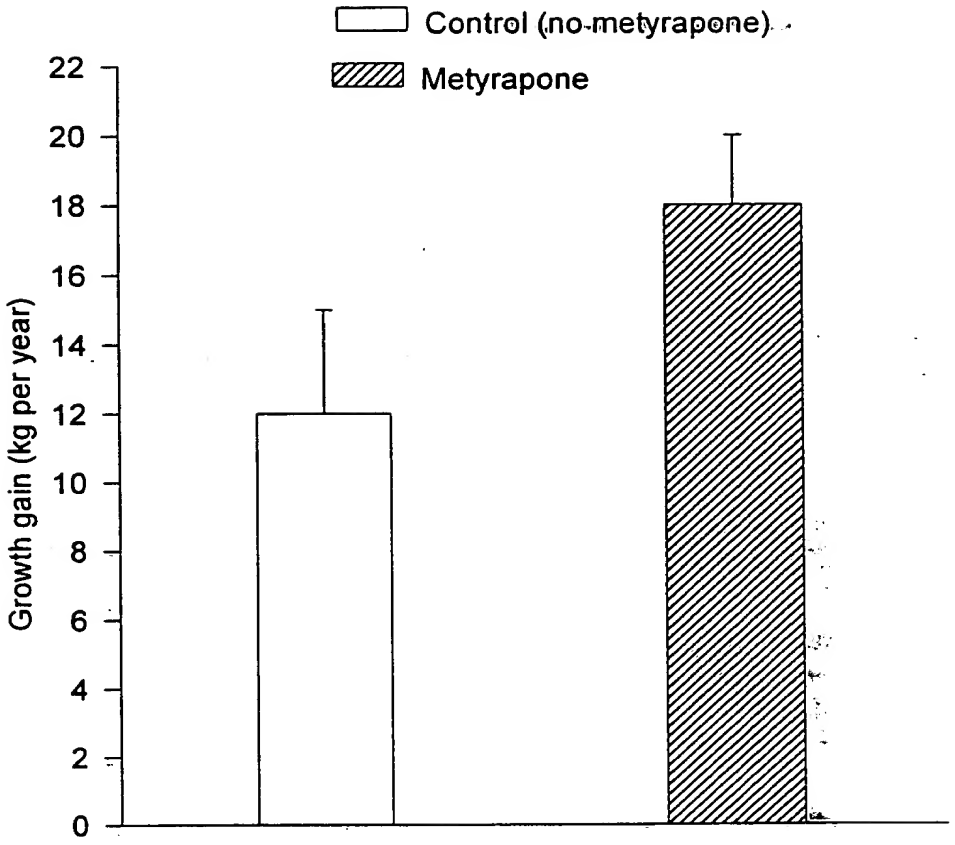


Figure 5


Control (no-metyrapone)
Metyrapone
Growth gain (kg per year)
22
20
18
16
14
12
10
8
6
4
2
0